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RESEARCH

Cyclooxygenase-2 – An Imperative Prognostic Biomarker in Oral Squamous Cell Carcinoma- An Immunohistochemical Study

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Abstract Oral squamous cell carcinoma (OSCC) is the most common head and neck squamous cell carcinoma (HNSCC) with metastasis and tumor recurrence resulting in 90 % of cancer associated mortality. COX-2, an inflammatory biomarker, has been shown to play a significant role in tumorigenesis of OSCC. To study the expression of COX-2 in OSCC by immunohistochemistry and investigate its association with the clinicopathological parameters including patient survival. A cross sectional study was carried out in 75 histologically confirmed cases of OSCC. COX-2 expression was evaluated by indirect streptavidin biotin method. The expression was semi-quantitatively assessed using established criteria. The expression profile of COX-2 was correlated with the clinicopathological details like tumor size, regional lymphnode metastasis, distant metastasis, clinical stage, local recurrence of tumor, histological grade, and survival of patient. Chi square and Kaplan Meier statistical tests were applied for assessing this association. COX-2 expression was absent in normal oral mucosa. Over expression of COX-2 was seen in 58 out of 75 specimens of OSCC. Overexpression of COX-2 was significantly associated with the lymphnode involvement, histological grade, local recurrence of tumor and patient survival. COX-2 expression represents an important biomarker of prognostic significance that may be used to identify a subset of patients at high risk and to predict patient survival.

Keywords Carcinoma · Squamous cell · Cyclooxygenase 2 · Immunohistochemistry

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Introduction

Oral cancer or oral cavity cancer, a subtype of head and neck cancer, is any cancerous tissue growth located in the oral cavity [1]. A common but troublesome neoplasm, oral cancer is the most devastating disease causing a significant disfigurement of the patient with severe morbidity and mortality. Oral cancer consistently ranks as one of the top ten cancers worldwide, with broad differences in geographic distribution, representing approximately 5 % of cancers in men and 2 % in women. India has one of the highest incidences of oral cancer in the world [2], with Oral squamous cell carcinoma (OSCC) representing more than 90 % of all oral cancers. Most cancer patients die from metastases and tumour recurrences rather than from their primary tumours; therefore, it is critical to study the molecular mechanisms of oral carcinogenesis and elucidate therapeutic targets to prevent the growth and spread of oral cancer. Cyclooxygenase (COX), officially known as prostaglandin-endoperoxide synthase (PTGS), is the key regulatory enzyme responsible for formation of important biological mediators, prostanoids; including prostaglandins, prostacyclin and thromboxane. Of the two isoforms of COX, COX-1 mediates many of the 'housekeeping' effects. Nearly all normal tissues express COX-1, with low to undetectable levels of COX-2. COX-2 is an inducible enzyme, becoming abundant in activated macrophages and other cells at sites of inflammation [3]. However, recently cyclooxygenase-2 has been seen to be over expressed in most malignant lesions,

including head and neck cancer, but has not been established yet in oral cancer. Considering this, a cross-sectional study was conducted to evaluate the immunohistochemical expression and distribution of COX-2 in different histological grades of OSCC and to correlate these findings with the clinicopathologic features in OSCC.

Thus, the current study aimed to determine the role of COX-2 in tumorigenesis of OSCC, and to substantiate if COX-2 could serve as a potential biomarker of tumor evaluation, including prognosis.

Materials and Methods

Materials

Institutional Ethics Committee (IEC) approval was obtained to carry out this study. The material for the present study included a total of 90 cases, including 75 cases of primary squamous cell carcinoma of bucco-alveolar mucosal complex and floor of the mouth diagnosed in the period between 2003 and 2009. Fouteen cases of dysplasia and ten healthy oral mucosal tissues were included in the study. Staging of primary carcinoma cases was done according to the TNM classification proposed by the Union for International Cancer Control (UICC). Formalin fixed paraffin embedded (FFPE) tissues preserved in the departmental archives were used for the work.. Relevant clinicopathologic details including the tumor staging, the histologic grading, and the development of recurrence or metastasis were given due consideration for all the cases. An analytical study was carried out to evaluate the expression of COX-2 in each of these cases by immunohistochemistry (IHC). Tissue sections obtained from colon carcinoma were taken as positive controls for COX-2. Endothelial cells constituted internal control.

All the cases were selected on the basis of strict inclusion and exclusion criteria. Only the cases which were histologically confirmed cases of OSCC of the bucco-alveolar complex and the floor of the mouth and the patients in whom the treatment (radiotherapy or chemotherapy) had not begun at the time of initial diagnosis and with complete clinicopathologic data and follow up available were included. Other proliferative lesions like proliferative verrucous leukoplakia, verrucous carcinomas were excluded.

The antibodies used for immunohistochemical staining were obtained from Leica Biosystems, Newcastle Ltd. Primary antibody: Novocastra™ Mouse Monoclonal Antibody Cyclooxygenase-2: NCL - COX-2 - 4H12 clone lyophilized tissue culture supernatant containing 15 mM sodium azide, 1 mg of COX-2 reconstituted with 1 mL of sterile distilled water. Secondary IHC Novolink Polymer Detection System containing: Peroxidase Block – 3 % Hydrogen Peroxide; Protein Block – 0.4 % Casein in phosphate-buffered saline, with stabilizers, surfactant and 0.2 % Bronidox L as a preservative; Post Primary Block – Polymer penetration enhancer containing 10 % (v/v) animal serum in tris-buffered saline/0.09 % ProClin™ 950; Secondary antibody Novolink polymer Anti-mouse IgG-Poly-HRP (each at 8 μg/mL) containing 10 % (v/v) animal serum in tris-buffered saline/0.09 % ProClin™ 950. Substrate: DAB Chromogen – 1.74 % w/v 3, 3′ – diaminobenzidine, in a stabilizer solution; NovoLink™ DAB Substrate Buffer (Polymer) – Buffered solution containing 0.05 % hydrogen peroxide and preservative. Haematoxylin: 0.02 % Mayer's Haematoxylin.

Methods

4 μm thick sections obtained from the FFPE tissue blocks were taken on uncoated slides and were stained with hematoxylin and eosin stains. All these cases were histologically graded using the border's criteria [4] which yielded us with 25 cases each of well, moderately and poorly differentiated carcinomas. Among the dysplastic cases, there were fourteen cases. Immunohistochemical staining was carried out on identical tissue sections taken on slides coated with 3-aminopropyl triethoxy silane (APES, Sigma – Aldrich Co. St. Louis, USA) and subjected to immunohistochemical staining using mouse anti-COX-2 monoclonal antibody, diluted at 1:100. For negative control the immunohistochemical staining was carried out in a similar manner but with the primary antibody replaced with tris-buffered saline (TBS).

The immunoreactivity of COX-2 was cytoplasmic. Positive cells were evaluated in 5 representative fields at 40x magnification. A total of 500 cells were examined. The distribution of antibody expression was assessed in the tumor cells.

Semiquantitative analysis was done to detect the intensity of COX-2 expression in the tumor cells by the method suggested by Itoh et al. [5]. The stained tissue sections were viewed under high power (40x) by a light microscope (OLYMPUS BLX4). A positive cell demonstrated a diffuse brown signal in the cytoplasm, independent of its intensity. To eliminate any inter observer bias the scoring was carried out self-reliantly by two observers.

The degree of staining was scored as follows: absent (−)= no staining in tumor cells; low (+1)=less than 5 % tumor cells with COX-2 staining; moderate (+2)=5–30 % tumor cells with COX-2 staining; diffuse (+3)=more than 30 % tumor cells with COX-2 staining, based on the criteria put forth by Itoh et al.[5]. COX-2 expression was then correlated to clinicopathological parameters including primary tumor size, stage of tumor, lymphnode metastasis, distant metastasis, tumor recurrence, histological grade of the tumor, and patient survival.

Statistical analysis was carried out using SPSS (statistical package for social service) version 16.0 for windows. Chi-square test was applied to study the correlation between COX-2 staining and the clinicopathological parameters. *P*

value<0.05 was considered significant for all statistical analysis. Kaplan-Meier analysis was done subsequently to correlate the COX-2 expression with the duration of survival of the patients.

Results

Immunohistochemical staining for COX-2 expression was carried out on all the tissues of OSCC using indirect streptavidin biotin immunoperoxidase technique. The immunohistochemical reaction was considered positive when a diffuse brown staining could be elicited in the cytoplasm of the tumor cells. The histological section of colon carcinoma stained with COX-2 was used as a positive control and confirmed against a negative control. The endothelial cells of the blood vessels were used as internal positive control (Fig. 1). The COX-2 positive staining in different grades of OSCC are depicted in Fig. 2a to c.

Out of the 10 normal oral mucosal tissues which were used as control group none of these were positive for COX-2 in the cytoplasm of the epithelial cells (Fig. 3). Among the fourteen cases of dysplasia, 3/14 (21 %) did not express COX-2 (Fig. 4), 2/14 (14 %) showed a moderate expression (Fig. 5) and 9/14 (64 %) cases showed a diffuse (+3) expression of COX-2 (Fig. 6).

In the test group which included OSCC cases, positive COX-2 expression in the cytoplasm was observed in all 75 cases (100 %). 17/75 (22.67 %) cases showed weak/ low expression of COX-2, 22/75 (29.33 %) cases showed moderate COX-2 expression and 36/75 (48 %) cases showed diffuse/ strong COX-2 expression. +2 and +3 together were considered as overexpression of COX-2.

Statistically significant correlation was observed between the status of the lymph node and COX-2 expression, with a p value of 0.032, as shown in Table 1. A statistically significant correlation with a p value of 0.006 was seen between the histological grades of OSCC and COX-2 expression, depicted in Table 2. P value of 0.098 was obtained showing the correlation between recurrence of the tumor and COX-2 expression very close to the level of significance, as presented in Table 3. Survival of the patient was compared with COX-2 expression and it was observed that 12/38 (31.57 %) cases that were alive without disease (AWOD) showed weak COX-2 expression and remaining 26/38 (68.43 %) of them were strongly positive for COX-2. Among the patients who were alive with the disease (AWD), 3/22 (13.63 %) cases showed low expression of COX-2 and 19/22 (86.37 %) of them showed COX-2 overexpression. 2/15 (13.33 %) cases who died of the disease (DOD) showed weak expression of COX-2 and 13/15 (86.67 %) cases among them showed overexpression of COX-2. No statistically significant correlation

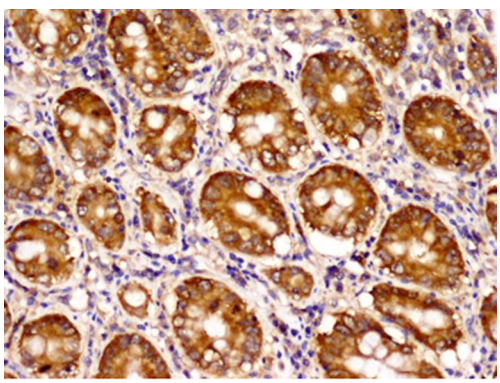

Fig. 1 Photomicrograph of the endothelial cells of blood vessel positive for COX-2 staining used as an internal control (IHC 10X)

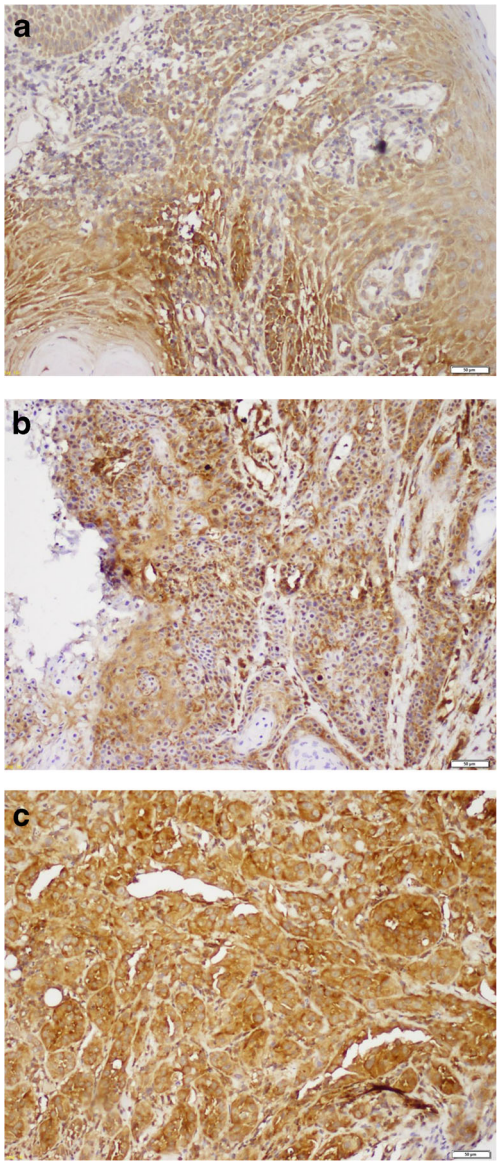

Fig. 2 **a** Photomicrograph of OSCC with good grade showing positive COX-2 expression (IHC 20X). **b** Photomicrograph of OSCC with moderate grade showing positive COX-2 expression (IHC 20X). **c** Photomicrograph of OSCC with poor grade showing positive COX-2 expression (IHC 20X)

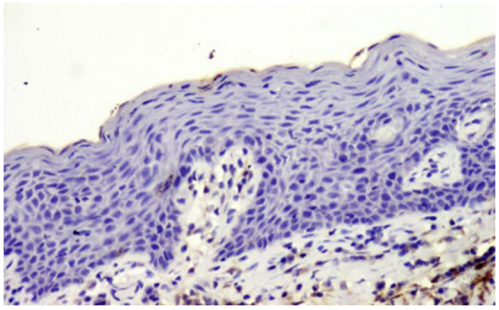

Fig. 3 Photomicrograph of the normal buccal mucosa showing the absence (−) of COX-2 expression (IHC (a) 10X)

could be derived between the survival of the patient and COX-2 expression as shown in Table 4. Subsequently, Kaplan-Meier analysis was done to correlate the COX-2 expression with the duration of survival of the patients. Death due to disease in patients with low COX-2 expression was seen to be significantly lower than those with COX-2 overexpression, as shown in Fig. 7.

Discussion

Oral cancer is one of the major cancers worldwide with a considerably lower overall five-year survival rate that has not significantly changed during the last two decades [6]. Although oral cancer is amenable to early diagnosis and treatment, the mortality rate has been consistently high, approximately 90 %, due to the failure to control the tumor recurrence and metastasis. A plethora of data specifies oral cancer to be a multistage genetic and epigenetic disease. Thus, the development of a molecular marker that is clinically applicable for detection, prognostication and therapy monitoring is strongly recommended.

It is recognized that one of the earliest reactions in any cancer is the inflammatory response. However, the role of

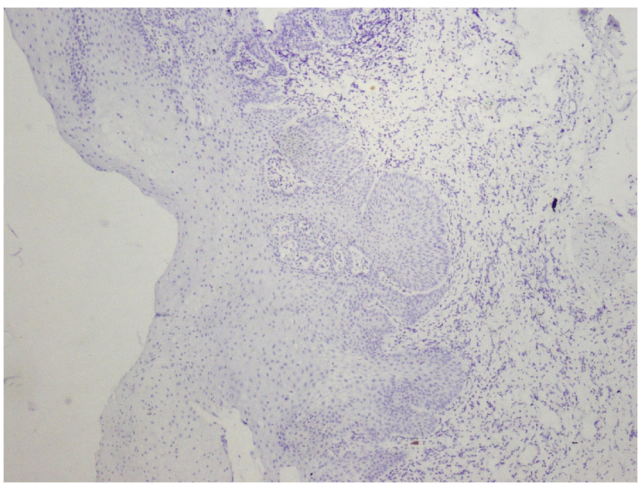

Fig. 4 Photomicrograph showing the absence (−) of COX-2 expression in dysplastic epithelium (IHC (a) 10X)

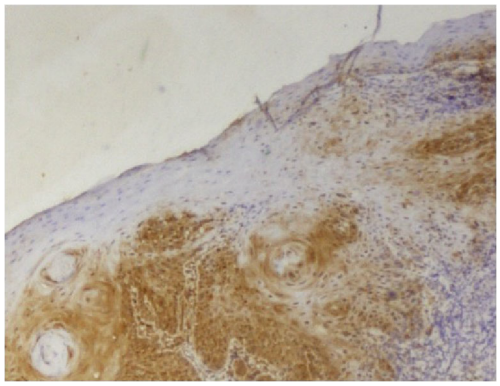

Fig. 5 Photomicrograph showing COX-2 expression in the basal and suprabasal layers of the dysplastic epithelium (IHC (a) 10X)

inflammation in oral cancer has been a topic of debate, as it is seen to have different roles. It was assumed to be beneficial as it indicates the host's response to oral cancer, thus attempting to subdue the lesion. Conversely, inflammation has been demonstrated to have tumor promoting potential as well. Hence, the need of the hour is to recognize the modulators of inflammation that might provide information of specific molecular targets. This can help in developing novel therapeutic modality against cancer.

COX-2, one of the isoforms of cyclooxygenase, is an inducible enzyme which is considered to be one of the chief mediators in the process of inflammation. COX-2 has been paid attention to because it could play an important role in the initiation and progression of carcinomas of various organs [7–11]. Up-regulation of COX-2 enhances the synthesis of prostaglandins, which causes increased proliferative activity of neoplastic cells, promotes angiogenesis [12], and inhibits immune surveillance [10]. Additionally, immunohistochemical overexpression of COX-2 depicts inhibition of apoptosis [13], and enhanced invasiveness [14]. More recently, a couple of reports that described about up-regulation or overexpression of COX-2 in squamous cell carcinomas of the head and neck have been published [15, 16]. However, it is still controversial whether or not it could be a prognostic factor for the patients with carcinoma of the head and neck and more

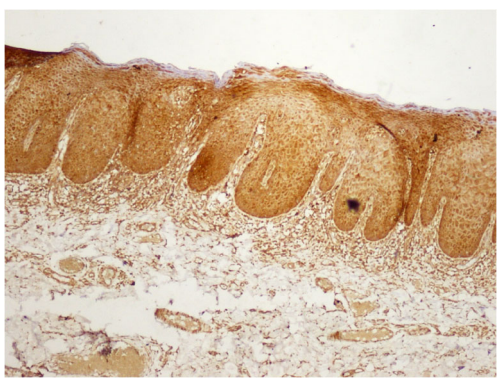

Fig. 6 Photomicrograph showing COX-2 expression through out the dysplastic epithelium (IHC (a) 10X)

Table 1 Showing the association between COX-2 expression and lymph node involvement

Lymph node involvement	Score	+1	+2 & +3	Total	Chi-square	*P* value
No	Count	14	31	45		
	% within node	31.11 %	68.89 %	100 %		
Yes	Count	3	27	30	4.577	0.032*
	% within node	10 %	90 %	100 %		
Total	Count	17	58	75		
	% within node	22.67 %	77.33 %	100 %		

* = statistically significant

specifically for oral cancer patients. The current study was hence conducted in an attempt to understand the role of COX-2 in carcinogenesis and its use as a prognostic marker for OSCC.

Along with OSCC cases, suitable control specimens of normal oral mucosal tissue specimens were included for comparison of COX-2 expression. The expression of COX-2 was seen to be absent in all the 10 specimens of normal oral mucosa, a finding similar to that made by Segawa et al. [17] and Mauro et al. [18]. However, among the 14 cases of dysplastic lesions a majority of the cases expressed COX-2, which is in line with the suggestion made by Mauro et al. (18) that the expression progresses from normal to dysplasia to carcinoma.

Most tissues do not routinely express COX-2 constitutively, as COX-1. It is only in the central nervous system [19] and seminal vesicles [20] where COX-2 has been demonstrated to be expressed normally. However, the stimulation of COX-2 in sarcoma (Src)-transformed fibroblasts [21], endothelial cells and monocytes treated with the tumor promoter tetradecanoylphorbol-acetate [22] or lipopolysaccharide create a notion that COX-2 is an inducible enzyme that produces prostaglandins during inflammatory and tumorigenic settings, thus supporting the finding in the present study, where it was not demonstrated in normal buccal mucosa.

The immunoreactivity of COX-2 was present to be in the form of cytoplasmic staining in the tumor cells of the OSCC cases. This finding was in similar lines with several studies where the expression of this biomarker was studied [5, 17, 23–25]. This observation suggests that upregulation of COX-2 may have an important role to play in the progression of OSCC. In our study, the immunoexpression of COX-2 was seen in all 75 cases (100 %) of OSCC, which is closely similar to the results observed by Li et al. [23], Soland et al. [26] and Nagatsuka et al. [24].

In this study it was seen that in addition to carcinoma cells, COX- 2 was expressed in stromal cells including macrophages, lymphocytes, some of the neutrophils, fibroblasts and vascular endothelial cells. These observations have also been made by Itoh et al. [5] in OSCC, suggesting that the immunoreactivity for COX-2 may be modulated by interaction of the stromal cells with the cancer cells in the process of tumor invasion.

It is well known that cancer cell development and survival is a multifactorial process, involving genetic mutation of normal cells as well as physiological changes within both cancer cells and also the body's defence mechanisms [27]. Immune response to cancer cell development and progression is of particular importance as it might play a potential role in tumor formation. Unresolved immune responses, such as chronic inflammation, can promote the growth and progression of cancer. The immune cells and the cellular factors produced from them, including both immunosuppressive and inflammatory cytokines, play dual roles in promoting or discouraging cancer development, and their ultimate role in cancer progression may rely heavily on the tumor microenvironment and the events leading to initial propagation of carcinogenesis [28].

COX-2/PGE2 pathway has been demonstrated to influence every hallmark of cancer, including oral cancer. Apoptosis, the

Table 2 Showing the association between COX-2 expression and the histological grade of tumor

Grade	Score	+1	+2 & +3	Total	Chi-square	*P* value
Well	Count	4	21	25		
	% within grade	16 %	84 %	100 %		
Moderate	Count	2	23	25		
	% within grade	8 %	92 %	100 %	10.193	0.006*
Poor	Count	11	14	25		
	% within grade	44 %	56 %	100 %		
Total	Count	17	58	75		
	% within grade	22.67 %	77.33 %	100 %		

* = statistically significant

Table 3 Showing the association between COX-2 expression and tumor recurrence

Recurrence	Score	+1	+2 & +3	Total	Chi-square	*P* value
No	Count	16	44	60		
	% within recurrence	26.67 %	73.33 %	100 %		
Yes	Count	1	14	15	2.738	0.098*
	% within recurrence	6.67 %	93.33 %	100 %		
Total	Count	17	58	75		
	% within recurrence	22.67 %	77.33 %	100 %		

* = statistically close to significant

process of programmed cell death, is a critical mechanism by which metazoan organisms control cell number, where selective cell suicide enables the efficient removal of superfluous, damaged or infected cells. COX-2/PGE2 pathway has been suggested to play a role in suppression of apoptosis, via activation of the Ras-mitogen activated protein kinase (MAPK/ERK) pathway [29].

Disturbances to normal tissue homeostasis that shift the balance from a state of equilibrium towards increased cell growth will invariably lead to the appearance of a neoplastic population of cells. The archetypal example of such an anti-proliferative signal is the soluble signaling factor transforming growth factor-beta (TGFb), which blocks progression through the G1 phase of the cell cycle via the suppression of c-Myc and activation of cyclin-dependent kinase inhibitors such as p15Ink4B and p21Cip1. COX-2/PGE2 pathway prevents the receipt of anti-growth signals since over-expression of COX-2 has been reported to cause down-regulation of the TGFb type II receptor [13].

The intrinsic capacities for self-renewal and limitless replicative potential are characteristics thought to be shared by stem cells and cancer cells [30]. Because of these apparent similarities, it has been proposed that cancer arises from the deregulation of pathways that maintain the stem/progenitor cell phenotype in a given tissue [31]. The COX-2/PGE2 pathway, by enhancing cell survival and growth, serves to assist the cells for acquisition of further cellular alterations that contribute to immortalization and the progression towards the full malignant phenotype.

In the course of solid tumor development, it is well recognized that the avascular tumor mass becomes dependent on angiogenesis for maintenance and progression, leading to angiogenic switch. Over-expression of COX-2 induces the production of angiogenic factors such as VEGF and basic fibroblast growth factor, which are instrumental in stimulating the formation of new blood vessels – a requirement for tumors should they wish to develop beyond a few millimeters in size [32]. The mechanism through which COX-2 might promote tumour vascularization is via the production of PGE2 and prostaglandin I2. These factors have been shown to participate in inducing endothelial cell dispersion and migration by integrin αVb3-mediated activation of the small guanosine 5′-triphosphatases Cdc42 and Rac [33].

Age, gender, clinical presentation, as well as the site of tumor presentation was not seen to be correlating with COX-2 expression in the current study. Also, no connotation was found in our study between the COX-2 level and the clinical stage and primary tumor size of the tumor. In contrast, Yoshimura et al. [34] advocated that COX-2 inhibition may lead to inhibition of tumor proliferation, indicating that COX-2 level is closely associated with tumor size. In vitro experiments have revealed that cells over-expressing COX-2 undergo phenotypic changes that could enhance their tumorigenic potential, such as exhibition of an increased adhesion to extracellular matrix proteins and resistance to apoptosis. This proliferative activity of COX-2 is believed to be primarily mediated by PGs.

Table 4 Showing the association between COX-2 expression and the patient survival

Survival	Score	+1	+2 & +3	Total	Chi-square	*P* value
AWOD	Count	12	26	38		
	% within survival	31.57 %	68.43 %	100 %		
AWD	Count	3	19	22		
	% within survival	13.63 %	86.37 %	100 %	3.491	0.175*
DOD	Count	2	13	15		
	% within survival	13.33 %	86.67 %	100 %		
Total	Count	17	58	75		
	% within survival	22.67 %	77.33 %	100 %		

AWOD alive without disease, *AWD* alive with disease, *DOD* died of disease; *=statistically not significant

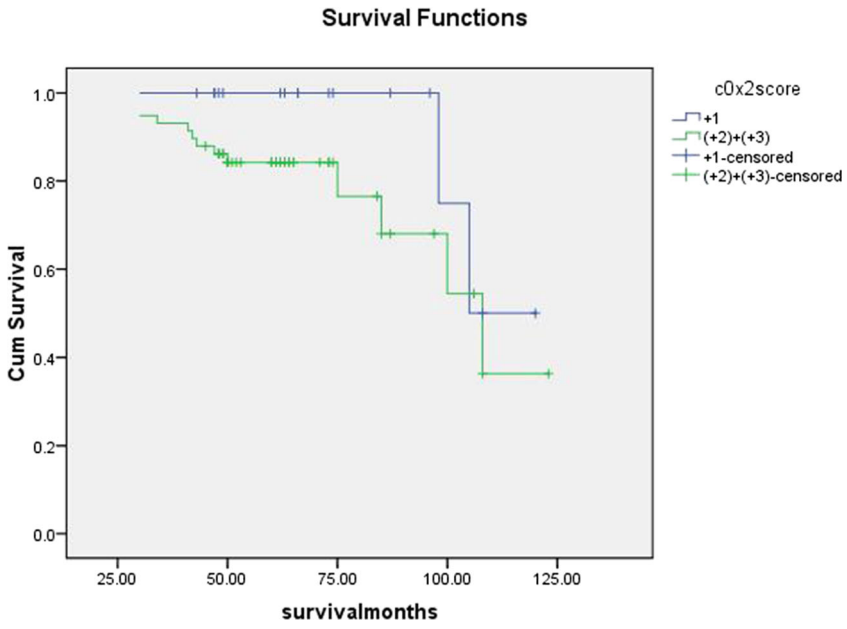

Fig. 7 Survival curve showing significant difference in death due to disease in patients with low expression of COX-2 and those with COX-2 overexpression

In the present study, a significant correlation was observed between the COX-2 overexpression and lymph node metastasis (p value=0.032), wherein 27/30 (90 %) patients with lymphnode metastasis were seen to be demonstrating COX-2 overexpression. This finding was in agreement with that by Itoh et al. [5]. The expression of COX-2 has been significantly correlated with lymphnode metastasis in prostate carcinomas [34], uterine cervical carcinomas [35] and gastric cancers. However, Lipari et al. [36] could not identify any significant association between COX-2 expression and salivary gland tumors. Li et al. [23], Sappayatosok et al. [37], Wang et al. [38] and Morita et al. [39] validated COX-2 as a predictor of lymphnode metastasis in OSCC at various sites, a finding similar to that in the current study.

It is well established that the primary cause of cancer mortality is the formation of distant metastases, making the capacity of tumour cells to invade and metastasize which constitutes one of the most pertinent hallmarks of cancer from a therapeutic perspective. Over-expression of COX-2 can modulate the adhesive properties of cancer cells and increase matrix metalloproteinase activity to promote invasion [40]. PGE2 promotes cytoskeletal reorganization and increases cancer cell migration and invasion via PI3K signaling. The stimulation of invasion and motility by PGE2 is dependent on the intracellular Src-mediated transactivation of EGFR [41]. Furthermore, hepatocyte growth factor signaling, which is classically associated with loss of cell-cell contact (or scattering) and invasive growth, is also transactivated by PGE2 in an EGFR-dependent approach. COX-2, hepatocyte growth factor and β-catenin are co-expressed at the invasive front of tumor specimens [42], suggesting their interplay in tumorigenesis. COX-2 has been identified as one of the four key 'metastasis progression' genes, which collectively synergize to mediate both tumor development and metastasis to other organs [43].

Our study also showed a close association between high COX-2 expression and local recurrence of the tumor (p= 0.098), wherein 14/15 (93.33 %) cases which showed local tumor recurrence demonstrated COX-2 overexpression, which is in agreement with the finding by Itoh et al. [5]. COX-2 has been identified as a candidate marker for maintenance of head and neck cancer initiating cells. While it may be true that numerous different genes can become altered during the development of a tumor, it has also recently been proposed that all cancers arise and are maintained by the deregulation of a relatively small number of signaling pathways. COX-2/PGE2 pathway, by enhancing cell survival and growth, serves to prime the tumor cells for the acquisition of further cellular alterations that contribute to immortalization and thus contributes for tumor recurrence.

Till date, there have been some ambiguous opinions with regard to the association of COX-2 overexpression and the histological grade of the tumor. Poorly differentiated tumors in esophagus and larynx have been reported to be more frequently negative for COX-2 as compared with well to moderately differentiated counterparts[45]. In the present study, the tumor was graded as per the criteria proposed by Bryne et al. [4], at the invasive front and correspondingly, COX- 2 expression was evaluated at the deepest point of invasion, similar to the method followed by Itoh et al. [5] and Soland et al. [26]. All the 75 OSCC cases (100 %) displayed COX-2 expression at the invasive front, which was in discrepancy to the results observed by Soland et al. [26], where most of the tumors were observed to be COX-2 negative at the invasive front. A significant correlation was attained between the COX-2

overexpression and the tumor grade (p=0.006). This finding was in similar lines with that of Renkonen et al. [16] who stated that COX-2 overexpression was closely associated with histological grade in SCC of tongue, and also with that of Sappayatosok et al. [38] and Nagatsuka et al. [24]. In disparity, Itoh et al. [5] and Soland et al. [26] could not establish a correlation between tumor grade and COX-2 expression.

Although not statistically significant (p value=0.175), the overall survival rate was also seen to be closely associated with COX-2 overexpression. Subsequent evaluation of survival interval by Kaplan-Meier analysis revealed that there was a significant difference in the number of deaths due to disease in cases with COX-2 overexpression than in cases with COX-2 lower expression. Itoh et al. [5] in their study evidenced that COX-2 overexpression is an independent predictor for disease-free survival but not for overall survival. On the other hand, Gallo et al. [44] reported that tumors of head and neck with COX-2 overexpression have shorter disease-free survival and overall survival than those without it. They also verified the presence of lymph node metastasis, the extent of vascularization, COX-2 expression and the tissue level of PGE2 as critical factors for patient survival, as was observed in the current study. COX-2 has been endorsed as an independent prognostic factor for cancer specific survival in esophageal adenocarcinoma [45], metastatic colorectal carcinoma and non-small cell lung cancer. Li et al. [23] have suggested COX-2 to have a clinical value in assessing the prognosis of OSCC. We experienced patients who survived longer than have expected inspite of repeated episodes of tumor recurrence, which may have caused the discrepancy during statistical analysis of COX-2 overexpression on the disease-free survival and over-all survivals in the present study.

Taken together, the data from our study adds weight to the growing body of evidence that COX-2 can be used to identify the subgroup of patients at high risk of nodal involvement and recurrence, while serving as a potential independent biomarker in predicting the patient survival in OSCC cases. Unlike most of the studies, the current study shows a significant correlation of COX-2 expression with the tumor grade, taken at the invasive front.

The contribution of COX-2 in carcinogenesis is due to its involvement in several key mechanisms including the conversion of pro-carcinogens to carcinogens as a consequence of arachidonic acid metabolism, stimulation of cell growth, inhibition of apoptosis through p53 suppression and bcl2 induction, stimulation of VEGF and angiogenesis, promotion of invasion and metastasis via matrix metalloproteinases induction and immunosuppression by IL-10 induction.

Based on the facts that COX-2 plays a role in carcinogenesis through inflammatory pathway, COX-2 inhibition might be a potential therapeutic target for increasing the patient survival. COX-2 inhibitors like celecoxib have been demonstrated to significantly inhibit cell proliferation in human endometrial adenocarcinoma [25]. Furthermore, COX-2 inhibitors are also known to enhance the toxic action of anti-tumor drugs against cancer cells [46]. These data support the fact that COX-2 inhibition can consequently enhance patient survival and can be employed for better prognosis of patients suffering from OSCC.

In the present study, we examined the immunohistochemical expression profile of COX-2 in OSCC and in the normal oral mucosa. Expression of COX-2 exhibited significant relationship with invasive front tumor grade and lymph node metastasis. It was also appreciated to be closely associated with recurrence of the tumor and the patient survival.

COX-2 as a single factor or in permutation with the other histopathological factors may thus be a valuable marker in predicting the tumor recurrence, metastatic potential, patient survival and hence, the prognosis of OSCC cases. Although our results suggest that COX-2 could be a useful biologic predictor of cancer recurrence and tumor outcome, subsequent prospective, large-scale studies are required to sanction its significance and applicability in the tumorigenesis of OSCC.

With recent studies, it is progressively apparent that the crosstalk between the cancer cells and the neoplastic stroma is involved in acquiring the capability of invasive growth, metastasis and tumor recurrence. Further studies on the role of COX-2 expression might provide a more profound insight into the mechanism of carcinogenesis in oral squamous cell carcinoma

Conflict of Interest None

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